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A STUDY OF THE GRAM-NEGATIVE BACILLI OF RENAL INFECTIONS

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In a recent report on the bacteria found in pyogenic renal infections we¹ stated that 85% of 116 patients were infected with gram-negative colonlike organisms either in pure or mixed form. No attempt was made at that time to classify these organisms other than to determine that they belonged to the colon or closely allied groups.

Clinical observation indicates that there must be either a vast difference in the virulence of the various strains in the colon group or an equally marked difference in the susceptibility of individual patients. The contrast in the lesions produced and course of the infection between the staphylococcus infections and those of the colon group as a whole is well known. It may be that equally prominent and important differences exist between the infections produced by the various colon groups.

The purpose of this paper is a classification of 86 different strains of colon bacillus-like organisms by means of cultural, fermentative and serologic methods. These organisms were all secured by ureteral catheter from the kidneys and isolated by cultivation on blood-agar plates.

It is a mere coincidence that just half of the organisms studied belong to the true colon group while the other half does not comply with the described characteristics of true colon organisms, but approach very closely the paracolon organism described by Mackie.² The essential difference lies in the fact that this last group does not ferment lactose and alkalizes rather than acidifies milk.

The motility tests were made on hanging drops of suspensions in broth taken from 18-24 hour old cultures. The indol determinations

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¹ Jour. Am. Med. Assn., 1918, 70, p. 1444.

² Jour. Path. and Bacteriol., 1913, 18, p. 137.

were made on 4-day old cultures in sugar-free broth using the para-dimethylamid-benzaldehyd method, while the reactions on gelatin were read after 6 weeks' cultivation at room temperature.

The various sugars were made in 1% strength in Hiss serum water medium. Following inoculation the reactions were read after 48 hours' incubation at 37 C.

TABLE 1
CULTURAL CHARACTERISTICS OF GRAM-NEGATIVE BACILLI FROM CATHETERIZED SPECIMENS OF URINE FROM THE KIDNEY

Groups With Number of Strains		Motility	Litmus Milk	Gelatin	Indol Reaction	Dextrose	Lactose	Mannose	Mannite	Saccharose	Dulcitate	Raffinose	Morphology
Paracolon Type 43 Strains		+	—	—	—	+	—	+	*	—	—	—	Gram-negative, pleomorphic bacilli, with tendency to form vacuoles, especially after 24 hours old.
True Colon Type 43 Strains	A 15	—	+	—	+14 —1	+	+		*	—	+		Typical gram-negative short plump bacilli.
	B 12	+ 2 —10	+	+ 1 —11	+	+	+		*	+	+		
	C 9	+ 1 — 8	+	+ 1 — 8	+	+	+		*	—	—		
	D 7	+ 2 — 5	+	—	+ 5 — 2	+	+		*	+	—		

* Variable.

Organisms are classified in the true colon group when they acidify litmus milk, ferment dextrose, lactose and mannite, produce indol and have no action on gelatin. The motility of organisms of this group is seen to be variable while all other characteristics conform very closely to type, there being 2 strains that partially liquefied gelatin, but are so similar to the others in all other respects that they are classified as true colon bacilli and not as members of the proteus group. Attention is directed to the fact that not one of these 86 organisms is classified as a proteus bacillus, while the older literature and text books speak of proteus bacilli and colon bacilli with the same degree of emphasis, and as being common infecting organisms of the urinary tract.

The fermentative reactions vary markedly among members of the true colon group. This is brought out in Table 1, showing that even those having similar fermentative reactions may have different characteristics in other respects; however, such irregularities are seen to be uncommon.

For the sake of emphasis the true colon organisms are divided according to their fermentative ability into 4 groups as seen in Table 2.

TABLE 2
FORTY-THREE TRUE COLON ORGANISMS CLASSIFIED ACCORDING TO FERMENTATION REACTIONS

	Dextrose	Saccha- rose	Lactose	Mannite	Dulcitol	Number of Strains
<i>B. Coli Communis</i>	+	—	+	+	+	15 A.
<i>B. Coli-Communior</i>	+	+	+	+	—	12 B.
<i>B. Acidi-lactici</i>	+	—	+	+	—	9 C.
<i>B. Aerogenes</i>	+	+	+	+	—	7 D.

Columns marked A., B., C., and D. same as in Table 1.

The immunity reactions of bacilli belonging to the colon group show a striking contrast to the action of immune serum for related organisms as *B. typhosus* and *B. paratyphosus*. A serum immune to a particular strain of *B. typhosus* will agglutinate the great majority of all other typhoid strains with small variations in degree. On the other hand, serum immune to various *B. coli* groups have been found to exert little or no action on strains which correspond in fermentative reactions and other characteristics to those used for immunization. It has even been considered that the action of colon agglutinin is practically limited to the particular strains used for immunization.

Immune serum was obtained for 4 strains belonging to the para-colon and a like number for true colon organisms. The technic for obtaining immune serum is as follows:

Half-grown rabbits were injected intravenously with killed cultures of bacilli in salt solution in 2 series thus: First day 1/100, 2nd day 1/75, 3rd day 1/50 of a 24-hour growth of an agar slant in 2 cc suspension; 9th day 1/50, 10th day 1/25, and 11th day 1/10 of the same growth. On the 15th day the rabbits were killed and the blood collected. The serum was removed and placed in sterile bottles without preservative. In all 8 serums there was a specific agglutinin titer of 1:1,280 or higher which seemed sufficient to distinguish group agglutinins if any were present.

Dilutions of the serum to be tested were made in small tubes with salt solution; a salt solution control was used. Thick suspensions were made of the 24-hour growth of bacilli from plain agar. An amount of this suspension equal to the amount of fluid already in each tube was added, making the serum dilutions range from 1:20 to 1:1,280. The tubes were incubated for 3 hours at 37 C. and then allowed to stand at room temperature for 24 hours before the readings were made.

As previously stated the specific agglutinin titer for each antiserum was 1:1,280 or more. To ascertain whether or not nonspecific agglutinin was present, cross agglutination tests were made, using the 8

antisera and testing the activity of each against the 8 strains used for immunization.

Table 3 represents results of the cross agglutination experiments, using 4 antiparacolon serums and 4 anticoli.

TABLE 3
CROSS AGGLUTINATION WITH EIGHT MONOVALENT SERUMS OF HIGH TITER

Number of Strain and Group	Antiparacolon Seru : s				Anticolon Serums			
	285	170	277	271	143	211	257	281
Paracolon 285.....	1,280*	1,280	1,280	1,280	320	40	0	40
Paracolon 170.....	1,280	1,280	1,280	1,280	40	80	0	0
Paracolon 277.....	1,280	1,280	1,280	1,280	0	0	0	0
Paracolon 271.....	1,280	40	1,280	1,280	40	40	40	0
Colon 143.....	80	40	0	0	1,280	640	0	0
Colon 211.....	40	20	0	20	320	1,280	80	0
Colon 257.....	160	40	0	80	0	40	1,280	20
Colon 281.....	40	40	40	40	0	0	0	1,280

* The figures placed in columns under each antiserum express the highest dilution of that serum which will agglutinate the organism represented by the numbers in the first left hand column.

It is seen that any antiparacolon serum agglutinated all the paracolon organisms tested as readily as the organism used to produce the serum, each organism being agglutinated at 1 to 1,280 by each of the 4 antisera with one exception. This result is in accord with the usual statement that members of this particular group of organisms are closely related as far as their reactions to immune serum is concerned. In this respect they simulate *B. typhosus* and *B. paratyphosus*. The same antiparacolon serums have only little or no agglutinating power for members of the colon group. This condition varies for each colon strain, some strains being agglutinated in dilutions of 1:160, while there are other strains that appear not to be affected by the action of any of the antiparacolon serums.

In marked contrast to the action of antiparacolon serum is that of anticolon serum which has a quantitatively specific action. Whereas each serum agglutinates the homologous organism in dilutions of 1:1,280 in each instance, it has very little or no agglutinating power for the other organisms of the colon group. The highest nonspecific agglutination is 1:320, while in most instances no agglutination is shown whatsoever. The anticolon serums react against the paracolon organisms very weakly and irregularly, their action being not unlike the action of paracolon serum on colon organisms.

To determine whether all of the paracolon organisms are biologically closely related, as indicated in Table 3, it seemed pertinent to

investigate further the agglutinating activity of certain antiparacolon serums against a greater number of paracolon strains, thus tending to minimize the possibilities of coincidence. Therefore 2 antiparacolon serums were tested against the entire 42 paracolon strains. The result is given in Table 4.

TABLE 4
AGGLUTINATION OF PARACOLON ORGANISMS BY TWO MONOVALENT SERUMS OF SAME GROUP

Antiparacolon Serum	Serum Dilution	Number of Strains
No. 285.....	1,280	18
No. 170.....	1,280	
No. 285.....	640	4
No. 170.....	80	
No. 285.....	80	4
No. 170.....	640	
No. 285.....	160	17
No. 170.....	160	

The uniform response of members of this group to agglutination is not so pronounced as might be concluded from a less extensive investigation. From Table 3 it may be concluded that these organisms are very closely related in every way, while a more comprehensive analysis justifies the belief that this is also a heterogeneous group, as only 18 of the 43 strains tested reacted like the organism used to produce the immune serum; that is, agglutinated at a dilution of 1:1,280. The remaining 25 strains can be divided serologically into 3 groups as seen in Table 4. While the group is quantitatively heterogeneous it is noteworthy that the members are not unrelated, as in most instances some agglutination takes place in all strains tested.

It becomes apparent that all members of both the colon and paracolon groups are liable to vary in one or many characteristics, thus any 2 strains may be different biologically, but in other respects very closely related. With these facts in mind there would appear to be no grounds for vaccine therapy in these infections unless the specific infecting organism were used for immunizing purposes.

An analysis of the clinical phase of these infections fails to reveal any difference either in the symptomatology, the changes or the course of the infection produced by any distinct subdivision of this large group.

Of the two most chronic infections encountered, in neither one of which we were able to secure a bacteriologic cure, one was due to a member of *B. coli-communis* group and the other to a paracolon

bacillus. All other bacilli tested were influenced by treatment regardless of the type of infection. In the two infections mentioned it seemed that local renal conditions might have been responsible for the chronicity of the course, as one patient had a moderate degree of hydronephrosis and the other had passed a renal stone.

There appears to be no symptoms peculiar to any type of infection and there is no apparent disparity in the severity of the infections with reference to distinct groups. Therefore, from these observations it may be concluded that the chief factors to determine the course of an infection by the heterogeneous *B. coli* group lie in the local and general conditions of the patients and not in the type of bacillus producing the infection. More detailed and extended observations are, of course, necessary to substantiate the foregoing assertion.

CONCLUSIONS

In a series of 86 strains of gram-negative bacilli from renal infections but one-half were found to be true colon bacilli, the other half being paracolon organisms.

The true colon bacilli were found, by means of fermentative reactions, to be members of four distinct groups. This grouping bears no relation to specific antibody reaction, since an antiserum for any one organism does not react with equal vigor against other members of the same or different groups.

By means of agglutination tests it was demonstrated that the colon group is markedly heterogeneous, as a monovalent serum reacts almost specifically. The paracolon group, however, while more closely related serologically than the members of the colon group, is still a heterogeneous group, but many members of this group reacted exactly alike in every respect in which they were tested.

There is no apparent clinical similarity between any two infections by members of any one group or subgroup. The difference between the various infections seems to be due to local or general conditions of the patient and not to any particular gram-negative bacillus.